PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Efficacy and safety of intravenous immunoglobulin with rituximab versus rituximab alone in childhood-onset steroid-dependent and frequently-relapsing nephrotic syndrome: protocol for a multicentre randomized controlled trial
AUTHORS	Hogan, Julien; Perez, Aubriana; Sellier-Leclerc, Anne-Laure; Vrillon, Isabelle; Broux, Francoise; Nobili, Francois; Harambat, Jerome; Bessenay, Lucie; Audard, V; Faudeux, Camille; Morin, Denis; Pietrement, Christine; Tellier, Stephanie; Djeddi, Djamal; Eckart, Philippe; Lahoche, Annie; Roussey-Kesler, G.; Ulinski, Tim; Boyer, Olivia; Plaisier, Emmanuelle; Cloarec, Sylvie; Jolivot, Anne; Guigonis, Vincent; Guilmin-Crepon, Sophie; Baudouin, Veronique; Dossier, Claire; Deschênes, Georges

VERSION 1 – REVIEW

REVIEWER	Dr Elisabeth Hodson Cochrane Kidney and Transplant Centre for Kidney Research The Children's Hospital at Westmead Sydney, Australia
REVIEW RETURNED	21-Feb-2020

GENERAL COMMENTS	Hogan et al present a protocol for a multicentre randomized controlled trial (RITUXIVIG) in French Centres in which children with steroid dependent nephrotic syndrome (SDNS) or frequently relapsing nephrotic syndrome (FRNS) are randomized to either single dose rituximab + 5 monthly IV doses of intravenous immunoglobulin (IVIG) or single dose rituximab alone with the primary outcome of the number with first relapse within 24 months. RITUXIVIG was first posted on ClinicalTrials.gov on June 18, 2018. ClinicalTrials.gov records the actual starting date as April 3, 2019 and the estimated study completion date of April 3, 2022. These dates are not recorded in the protocol submitted to BMJ Open. Abstract: Children and young adults from 2-25 years will be included in whom the first episode of steroid sensitive nephrotic syndrome (SSNS) occurred below 18 years of age. Both steroid dependent (SDNS) and frequently relapsing (FRNS) children will be included. However the authors use SDNS to include both groups of children under methods though not introduction. The authors need to make it clear to readers of the protocol that both groups of children are to be included. Article summary: • Although paediatric nephrologists are well aware that rituximab use is limited to children with FRNS or SDNS, this is not made clear in the article summary and should be. • Intravenous administration of medications is both a strength and a weakness of the study because children in the experimental group

	(Rituximab + IVIG) will require 11 IV administrations of medications during the study based on the section on Procedures in the main section. Background: • I think that the Background is too long at four pages. The most important parts relate to rituximab and IVIG so the earlier parts should be shortened. • Again the authors use steroid-dependent to describe both FRNS and SDNS. This is confusing for the reader, who might assume that the RCT is limited to participants with SDNS. The authors report that 60% become steroid dependent whereas this number appears to refer to both SDNS and FRNS. • It is not strictly true that there are no guidelines for the treatment of SDNS/FRNS since the KDIGO guidelines published in 2012 cover this. The updated KDIGO guidelines will be circulated for comment soon, I understand. • The information on the use of IVIG in a wide range of autoimmune diseases and antibody mediated rejection in kidney transplants is valuable. Methods/Design: • Primary objective and secondary objectives: These are clearly stated • Study design: This is clearly stated • Eligibility criteria: Study inclusion criteria: I suggest separating point 3 into 2 points to cover SDNS separately from FRNS Study exclusion criteria: There is no section describing whether young people, who are sexually active, are required to use a reliable form of contraception The authors need to state what “protected adults” are since this is not a term used in many areas. • Outcomes: The definition of the primary outcome of relapse is clearly reported. • Procedures: I am not clear as to why the IVIG has to be given on two consecutive days particularly to small children and I would like to see an explanation included. Children in the IVIG group will have 10 infusions for IVIG as well as a single injection of rituximab. Presumably some children may have to stay in hospital overnight to receive the second infusion. This is a large burden for children and their families. • Adverse events: It is disappointing to see that this study does not plan to examine patient reported outcomes formally throughout the study and only reports on adverse effects when these occur and are reported to clinicians. In particular studies of a child’s behaviour using formal testing would be valuable as was done in the PREDNOS study. I would like to know why the triallists did not include this. • It is also disappointing to see that patients and carers were not involved in the design of the study. I would like to know why the triallists did not involve patients and carers in the study design.

REVIEWER	WILLIAM SMOYER Nationwide Children's Hospital; The Ohio State University; USA
REVIEW RETURNED	10-Mar-2020

GENERAL COMMENTS	This is a very interesting and potentially important study.

	The study is well-designed and likely to yield important new information to meet an important unmet medical need. A few suggestions below could improve the likelihood for success of the trial: 1 - Please publish your preliminary findings noted in the Introduction ASAP ! 2 - Secondary Objectives: Time to relapse will be important as it will require fewer patients to achieve significant results.. However, you will need to add a detailed home monitoring plan for first-AM urine dipsticks to be able to accurately determine time to relapse. Would also add percentages to your plans for absolute #s of relapses. 3 - Secondary Objectives: Would suggest you add significantly more details to the specific safety outcomes you plan to measure (i.e. infections requiring medical care, infections requiring hospitalization, infusion reactions, etc) and list a specific Safety Outcome Measures section just behind the Secondary Outcomes measures, since the safety of this treatment will be critical. 4 - Would separate SDNS criteria from FRNS criteria in Inclusion Criteria and clearly state that either group will be eligible... Do you want to ensure that a certain percentage of patients must have SDNS, or will you be content if all patients have FRNS?... I think you will want at least 30-40 % to have the more severe SDNS for your trial to have maximal value... 5 - Inclusion Criteria: For #4, I suggest you clarify first-AM urine samples and state specifically how long they must/can be in remission to be eligible... 6 - Exclusion Criteria - Would consider amending #2 to state " genetic mutations known to be associated with nephrotic syndrome"... Also, I suggest you add "presence of another ACTIVE glomerular disease" and consider adding "eGFR <60" as additional exclusion criteria. Lastly I suggest you modify #6 to improve clarity, such as saying "known CHF, LVH, or cardiomyopathy". 7 - I suggest you specify that all urine protein: creatinine ratios in the study be first-AM samples, to systematically exclude possible orthostatic proteinuria, as this could dramatically confound your results. 8 - I suggest you consider increasing your anticipated dropout rate to 10% from 5% to enhance the likelihood for successful completion of the trial. 9 - I suggest you clarify the sponsor for this study, and what type of organization it is... Is it connected in any way with the production or distribution of either study drug ? Will it benefit from a specific result of the trial?

REVIEWER	Abhik Das RTI International, USA
REVIEW RETURNED	13-Apr-2020

GENERAL COMMENTS	Overall, this is a competent trial protocol for a study that seeks to answer an important question. The statistical aspects of the protocol

	are not well addressed, however. It is unclear if randomization is not stratified by center for this multicenter study. No stratification factors for randomization are discussed. Sample size calculations are based on a chi square test comparing two independent proportions, while the analysis plan talks about a Kaplan Meier log rank test. These should be consistent. Comparison of secondary outcomes for number of events is confusingly based on a chi square test, whereas such a comparison is typically based on a Poisson test or a log transformed t test. Discussion of analysis plan is somewhat perfunctory and does not consider any adjusted analyses. How the multicenter nature of the study will be accounted for in the analysis is also not addressed. Issues of treatment heterogeneity and subgroup effects are also not considered. There is a long list of precise entry criteria for this trial. The investigators should consider whether this accurately reflects the population of patients that the new therapy will be applied to, if the trial is successful. The write-up uses non standard english in places and will benefit from the services of a competent scientific editor.

REVIEWER	LM Ho The University of Hong Kong
REVIEW RETURNED	18-Apr-2020

GENERAL COMMENTS	1. (p12, line 33) One of the secondary objectives is “to compare the tolerance and safety of the two strategies”. Please describe how tolerances can be compared between the two groups. 2. (p13, lines 15 & 49) Based on the selection criteraia, the subject can be a bady (2 years old) or an adult. It is possible that there may be a substantial variation in age. If the age is not successfully randomized, (eg mean age of one group is much greater than the other group), whether this can confound the results? Is there any restriction on age? 3. (p13, line 42; p14 line 5), What is the rationale to exclude patients with no medical insurance and protected adults from the study? 4. (p14,line 40) typo: patient [ia] randomized 5. (p15, line 3) What is the block size of the permuted block randomisation? As this is a multicentre trial, are subjects from different centres comparable? Will the block randomisation take into account multicentre, so that the numbers of subjects per group are the same in each centre? If not, will this imbalance be a source of selection biases? 6. (p18, line 3) Intention to treat approach is adopted for data analysis. How to deal with missing data, eg the number of relapses and adverse events, cumulative doses of steroid? 7. (p18, line 21) “Comparison of the number of relapses and the number of adverse events between the groups will be performed using a Chi-square test”. My understanding is that each patient can have more than one relapse and more than one adverse event. How can chi-square test be used to compare the number of relapses (or adverse events) between the two arms? Moreover, the numbers of relapses (or adverse events) will be underestimated for those who leave the study during the follow-up. How this can be handled? 8. (p18, line 25), why the non-parametric test (Mann-Whitney test) will be used for analyzing cumulative doses, instead of parametric tests? 9. (p18, line 12) Kaplan-Meier method will be used to describe the

	risk of relapse, which requires data on time to event (ie time to relapse). But why “a similar method will be used to study the time to first relapse”(line 18)? 10. (p19, line 13) The study was approved by the Ethics committee on May 17, 2018. Please state whether the study is ongoing? If it is ongoing, what is the date of the study?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Hogan et al present a protocol for a multicentre randomized controlled trial (RITUXIVIG) in French Centres in which children with steroid dependent nephrotic syndrome (SDNS) or frequently relapsing nephrotic syndrome (FRNS) are randomized to either single dose rituximab + 5 monthly IV doses of intravenous immunoglobulin (IVIG) or single dose rituximab alone with the primary outcome of the number with first relapse within 24 months. RITUXIVIG was first posted on ClinicalTrials.gov on June 18, 2018. ClinicalTrials.gov records the actual starting date as April 3, 2019 and the estimated study completion date of April 3, 2022. These dates are not recorded in the protocol submitted to BMJ Open.

Because of delays in the opening of some centers and in patients’ inclusion, the inclusion period has been extended to 2 years. Given the 2-year follow-up in the trial, the expected completion date is now April 3, 2023. This has been added in the manuscript as requested:” *Inclusions started in April 2019 and are expected to be completed in April 2021. The expected study completion date is April 2023*.”

Abstract: Children and young adults from 2-25 years will be included in whom the first episode of steroid sensitive nephrotic syndrome (SSNS) occurred below 18 years of age. Both steroid dependent (SDNS) and frequently relapsing (FRNS) children will be included. However the authors use SDNS to include both groups of children under methods though not introduction. The authors need to make it clear to readers of the protocol that both groups of children are to be included.

We agree with the reviewer that our trial actually includes both SDNS and FRNS. We edited the manuscript to make this point clear to the readers.

Article summary:
• Although paediatric nephrologists are well aware that rituximab use is limited to children with FRNS or SDNS, this is not made clear in the article summary and should be.

We state in the abstract that: “*Several studies confirm that rituximab is effective in preventing early relapses in SDNS/FRNS, however the long-term relapse rate remains high (~70% at 2 years)*” to clarify this point.

• Intravenous administration of medications is both a strength and a weakness of the study because children in the experimental group (Rituximab + IVIG) will require 11 IV administrations of medications during the study based on the section on Procedures in the main section.

We agree that there is a real burden associated with the administration of IVIG. This is also why we are interested a major (>30% reduction in absolute relapse rate, RR=0.5) and sustained (at least 2 years) benefit of this strategy.

Background:
• I think that the Background is too long at four pages. The most important parts relate to rituximab and IVIG so the earlier parts should be shortened.

We agree with the reviewer and shortened the introduction as requested.

• Again the authors use steroid-dependent to describe both FRNS and SDNS. This is confusing for the reader, who might assume that the RCT is limited to participants with SDNS. The authors report that 60% become steroid dependent whereas this number appears to refer to both SDNS and FRNS.

We agree with the reviewer and edited the manuscript to clearly specify that this rate accounts for both SDNS and FRNS:" *However, 60% will become steroid-dependent or frequent-relapsers with a major risk of morbidity related to the complications of the relapses.*"

• It is not strictly true that there are no guidelines for the treatment of SDNS/FRNS since the KDIGO guidelines published in 2012 cover this. The updated KDIGO guidelines will be circulated for comment soon, I understand.

We edited the manuscript to indicate that there is currently no consensus on the treatment of SDNS/FRNS and that the KDIGO guidelines only list potential steroid-sparing agent without giving indication which to prefer as follow:" *There is currently no consensus on the treatment of SDNS/FRNS and KDIGO guidelines only list potential steroid-sparing agent withot giving indication which to prefer*."

• The information on the use of IVIG in a wide range of autoimmune diseases and antibody mediated rejection in kidney transplants is valuable.

We thank the reviewer for this positive comment.

Methods/Design:
• Primary objective and secondary objectives: These are clearly stated

We thank the reviewer for this positive comment.
• Study design: This is clearly stated

We thank the reviewer for this positive comment.
• Eligibility criteria:
Study inclusion criteria: I suggest separating point 3 into 2 points to cover SDNS separately from FRNS

We edited the presentation of the inclusion criteria as suggested.

Study exclusion criteria: There is no section describing whether young people, who are sexually active, are required to use a reliable form of contraception.

A pregnancy test is perform before inclusion in the study in women of childbearing age and effective contraception will be given to these patients at inclusion. This contraception will be continued for one year after the last infusion of Rituximab. We know specify this in the exclusion criteria as suggested by the reviewer.

The authors need to state what "protected adults" are since this is not a term used in many areas.

Protected adults refers to adults under guardianship. We edited the manuscript accordingly.

• Outcomes: The definition of the primary outcome of relapse is clearly reported.

We thank the reviewer for this positive comment.
• Procedures: I am not clear as to why the IVIG has to be given on two consecutive days particularly to small children and I would like to see an explanation included. Children in the IVIG group will have 10 infusions for IVIG as well as a single injection of rituximab. Presumably some children may have to stay in hospital overnight to receive the second infusion. This is a large burden for children and their families.

The infusions were divided over 2 consecutive days to allow the slow administration of IVIG and improve tolerance. Although, different infusion modality exist, we decide to protocolize it in the trial to insure comparability.

• Adverse events: It is disappointing to see that this study does not plan to examine patient reported outcomes formally throughout the study and only reports on adverse effects when these occur and are reported to clinicians. In particular studies of a child's behaviour using formal testing would be valuable as was done in the PREDNOS study. I would like to know why the triallists did not include this.

We acknowledge this limitation of our study and that having data on PROs would have been valuable. However, unlike the PREDNOS study, which was a non-inferiority study, in our study the intervention in the experimental arm has a clearly higher burden for the patients with multiple IV infusions. Therefore, only a clear superiority of the experimental arm would motivate the use of IVIG in clinical practice and we anticipate that differences in PROs are less likely to inform treatment choice in this case.

• It is also disappointing to see that patients and carers were not involved in the design of the study. I would like to know why the triallists did not involve patients and carers in the study design.
We acknowledge that the involvement of patients and caregivers in the design of the study would have been valuable. We do not have a good reason for this and acknowledge the need for improvement in this area in France.

Reviewer: 2
This is a very interesting and potentially important study.
The study is well-designed and likely to yield important new information to meet an important unmet medical need.
We thank the reviewer for this positive comment.

A few suggestions below could improve the likelihood for success of the trial:

1 - Please publish your preliminary findings noted in the Introduction ASAP !

We agree with the reviewer on the need to publish these preliminary data. There publication is complicated by important bias in patient selection and lack of standardization of the treatment received as the regimen has been modified over time. We will do our best to analyze these data in the most informative way and to publish these results.

2 - Secondary Objectives: Time to relapse will be important as it will require fewer patients to achieve significant results. However, you will need to add a detailed home monitoring plan for first-AM urine dipsticks to be able to accurately determine time to relapse. Would also add percentages to your plans for absolute #s of relapses.

The primary objective of our study is to demonstrate a difference in the proportion of patients with at least one relapse within 24 months. We agree with the reviewer that the analysis of the time to relapse is likely to yield important results. The protocol plans for a weekly first-AM urine dipstick until 12 months after rituximab injection and once every two weeks between 12 and 24 months. All positive result will be confirmed by an uPCR measurement. Although, a daily check-up would have been ideal, we felt that this was a reasonable compromise between the precision of our measurement and the burden to the participants.

3 - Secondary Objectives: Would suggest you add significantly more details to the specific safety outcomes you plan to measure (i.e. infections requiring medical care, infections requiring hospitalization, infusion reactions, etc) and list a specific Safety Outcome Measures section just behind the Secondary Outcomes measures, since the safety of this treatment will be critical.

We agree with the reviewer and now specify the adverse events specifically monitored in this trial as follow:*" Other adverse events monitored during the follow-up include infections requiring*

hospitalization, infections not requiring hospitalization, Progressive multifocal leukoencephalopathy, Neutropenia, Acute kidney injury stage 3: increase in creatinine of > or = 200% or eGFR < or = 35ml/min/1.73 m2 (if age < 18 yr) if patients with previously normal renal function, allergic reaction ≥ grade 3 and infusion tolerance."

4 - Would separate SDNS criteria from FRNS criteria in Inclusion Criteria and clearly state that either group will be eligible... Do you want to ensure that a certain percentage of patients must have SDNS, or will you be content if all patients have FRNS?... I think you will want at least 30-40 % to have the more severe SDNS for your trial to have maximal value...

We agree with the reviewer that it is important to clearly differentiate SDNS from FRNS. We edited the manuscript o clearly reflect the inclusion of both categories in out trial. The inclusion in our trial are not stratified on patient status but we expect based on the epidemiology and the current practices in France that over 50% of the patients included will be SDNS.

5 - Inclusion Criteria: For #4, I suggest you clarify first-AM urine samples and state specifically how long they must/can be in remission to be eligible...

We decided not to require first-AM sample to confirm remission as we felt that a negative uPCR at any time of the day was enough to support remission. We agree that it would have been good to require a minimal time on remission before the inclusion. Although this was not the case, in practice the time between the diagnosis of remission, the information about the study and the scheduling of the RTX infusion results in several weeks of sustained remission (max 3 months) before initiation of the trial.

6 - Exclusion Criteria - Would consider amending #2 to state " genetic mutations known to be associated with nephrotic syndrome"... Also, I suggest you add "presence of another ACTIVE glomerular disease" and consider adding "eGFR <60" as additional exclusion criteria. Lastly I suggest you modify #6 to improve clarity, such as saying "known CHF, LVH, or cardiomyopathy".

We edited the exclusion criteria as suggested. As no eGFR cut-off was included in the protocol that is currently ongoing, we did not add it to the manuscript. However, we do not expect to include patients with significantly impaired eGFR.

7 - I suggest you specify that all urine protein: creatinine ratios in the study be first-AM samples, to systematically exclude possible orthostatic proteinuria, as this could dramatically confound your results.

We agree with the reviewer that orthostatic proteinuria could confound the results. Although it is usually common practice, we will remind the sites PIs to recommend first-AM samples. Of notes, any positive result is confirmed limiting the risk of false positive. As the trial is already ongoing, we did not formally include this requirement in the present manuscript.

8 - I suggest you consider increasing your anticipated dropout rate to 10% from 5% to enhance the likelihood for successful completion of the trial.

Based on previous national clinical trials performed in France, we do not expect our dropout rate to exceed 5%. Moreover, although we do want to maximize the likelihood of success of this trial, we do not want to extend the inclusion period to much.

9 - I suggest you clarify the sponsor for this study, and what type of organization it is... Is it connected in any way with the production or distribution of either study drug ? Will it benefit from a specific result of the trial?

The sponsor of the trial is a non-profit public organization supervising all the public hospitals in the Paris area. This trial is an investigator-initiated trial and neither the PI nor the sponsor have any connection with Pharma company that may benefit from the results of this trial. We clarified this in the manuscript as follow: "*The sponsor was Assistance Publique – Hôpitaux de Paris (Clinical Research and Innovation Department, a non-profit public organization supervising all the public hospitals in the*

Paris area"

Reviewer: 3

Overall, this is a competent trial protocol for a study that seeks to answer an important question.

We thank the reviewer for this positive comment.

The statistical aspects of the protocol are not well addressed, however. It is unclear if randomization is not stratified by center for this multicenter study. No stratification factors for randomization are discussed.

No stratification of the randomization by center was planned for this trial. We clarify this fact in the manuscript as follow:" *No stratification of the randomization was planned".*

Sample size calculations are based on a chi square test comparing two independent proportions, while the analysis plan talks about a Kaplan Meier log rank test. These should be consistent. Comparison of secondary outcomes for number of
events is confusingly based on a chi square test, whereas such a comparison is typically based on a Poisson test or a log transformed t test.

The primary outcome in this study is the occurrence of a relapse within 24 months after RTX injection. This outcome was chosen as primary outcome because of the possible difficulty to determine the precise date on relapse based on weekly urine monitoring and the need to insure the feasibility of the study by containing the number of participants to include. Indeed, based on our hypothesis aiming a demonstrating a reduction of the proportion of patients with at least one relapse from 60% to 30% at 24 months, the number of patients to include was estimated at 90 (using a chi square test) for a power of 80% and a two-sided type I error of 5%.

Time to first relapse was included as a secondary outcome. Assuming a similar reduction of 30% of relapse rate at 24 months in the rituximab + IVIg group, i.e. an HR at 0.5, a low number of lost to follow-up (2.5%) and a median survival time in the control group at 9 months based on previous studies, we expect to have a power of 70% to detect such difference with a two-sided type I error of 5%. Accordingly, KM and log-rank test will be used to compare time to first relapse.

We agree with the reviewer that number of relapses and the number of adverse events should be compared by a log-transformed t-test or a Mann-Whitney according to the distribution of the data and we edited the manuscript accordingly.

Discussion of analysis plan is somewhat perfunctory and does not consider any adjusted analyses. How the multicenter nature of the study will be accounted for in the analysis is also not addressed. Issues of treatment heterogeneity and subgroup effects are also not considered.

For the primary analysis of this study, we do not plan to adjust the result unless obvious differences between the study groups were found despite randomization. Also, given the high number of center (22 for 90 patients), we do not plan to include a center-effect in the analysis. However, we agree with the reviewers that the following subgroup analysis are of interest and will be conducted although the randomization was not stratified on any of these factors and we may be underpowered to detect a significant difference in a specific subgroup. Sub-group analysis will be performed based on INS status (SDNS/FRNS) and age.

There is a long list of precise entry criteria for this trial. The investigators should consider whether this accurately reflects the population of patients that the new therapy will be applied to, if the trial is successful.

Despite the long list of inclusion and exclusion criteria, the inclusion criteria in this study are very broad and the results of this study are likely to be broadly generalizable.

The write-up uses non standard english in places and will benefit from the services of a competent

scientific editor.
The manuscript has been reviewed by a native English speaker as requested.

Reviewer: 4

Please leave your comments for the authors below
1. (p12, line 33) One of the secondary objectives is “to compare the tolerance and safety of the two strategies”. Please describe how tolerances can be compared between the two groups.

We know include in the manuscript the list of adverse events specifically monitored in the trial: *Other adverse events monitored during the follow-up include infections requiring hospitalization, infections not requiring hospitalization, Progressive multifocal leukoencephalopathy, Neutropenia, Acute kidney injury stage 3: increase in creatinine of > or = 200% or eGFR < or = 35ml/min/1.73 m2 (if age < 18 yr) if patients with previously normal renal function, allergic reaction ≥ grade 3 and infusion tolerance.”*

2. (p13, lines 15 & 49) Based on the selection criteria, the subject can be a baby (2 years old) or an adult. It is possible that there may be a substantial variation in age. If the age is not successfully randomized, (eg mean age of one group is much greater than the other group), whether this can confound the results? Is there any restriction on age?

We agree with the reviewer that the age range eligible for the trial is broad. However, given the epidemiology of the disease, extreme ages will be rare. Moreover, we expect randomization to adequately distribute patients in the two groups. Moreover, we will perform a subgroup analysis based on age. Finally, regression models looking at independent predictors of relapse will be perform.

3. (p13, line 42; p14 line 5), What is the rationale to exclude patients with no medical insurance and protected adults from the study?

Under the French Universal Healthcare system all French citizen and legal immigrants have medical insurance. Therefore, this criteria only excludes illegal immigrants that would not be covered for the expenses related to usual care. Similarly, patients that are not able to give their informed consent cannot be included in clinical trials. This is a standard procedure according to French law.

4. (p14,line 40) typo: patient [ia] randomized

We thank the reviewer for carefully reviewing our manuscript. We edited the typo.

5. (p15, line 3) What is the block size of the permuted block randomisation? As this is a multicentre trial, are subjects from different centres comparable? Will the block randomisation take into account multicentre, so that the numbers of subjects per group are the same in each centre? If not, will this imbalance be a source of selection biases?

We used mixed blocks to perform the randomization in order to avoid the predictability of treatment group assignment given the non-blinded design of our trial. The block randomization did not take into account the centres but given the type of intervention, the biological nature of the outcome and the overall homogeneity of practice in France, we do not expect a major centre effect.

We now provide more details on the randomization method as follow: “*randomization will be performed using a web-based application and a secured access (CleanWeb®) in a 1:1 ratio […] according to a computer-generated list of randomly permuted blocks (mixed blocks)*”.

6. (p18, line 3) Intention to treat approach is adopted for data analysis. How to deal with missing data, eg the number of relapses and adverse events, cumulative doses of steroid?

The trial was designed to minimize the risk of missing data. However we acknowledge that missing data especially for some secondary outcomes are possible. We now added in the manuscript the plan to handle mising data as described in the protocol: “*In addition, quality control of the data is planned to detect missing and inconsistent data. All missing data will be sought in the patients’ medical*

records. If missing data cannot be recovered by the study monitors, a multiple imputation procedure based on a "missing at random" assumption will be considered"

7. (p18, line 21) "Comparison of the number of relapses and the number of adverse events between the groups will be performed using a Chi-square test". My understanding is that each patient can have more than one relapse and more than one adverse event. How can chi-square test be used to compare the number of relapses (or adverse events) between the two arms? Moreover, the numbers of relapses (or adverse events) will be underestimated for those who leave the study during the follow-up. How this can be handled?

We agree with the reviewer that there was inconsistency in the analytical plan presented that have been amended as follow:" *Comparison of the number of relapses, the number of adverse events and the cumulative doses of steroids over the study period will be performed using either a log-transform t-test or a Mann-Whitney test based on the distribution of the data.*"

In our study, all patients will undergo 24 months of follow-up even in the case of early relapse. Even in case of loss-to-follow up, because all pediatric nephrology centers are included in our study and have strong collaboration and because INS relapse systematically result in an encounter in one of these centers, we expect to be able to accurately collect the number of relapses for all patients.

8. (p18, line 25), why the non-parametric test (Mann-Whitney test) will be used for analyzing cumulative doses, instead of parametric tests?

We agree with the reviewer that the presence of 45 patients in each arm may allow the use of a parametric test such as a student t-test. We will assess the distribution of the cumulative steroid doses in our population and decide whether to use a parametric or a non-parametric test based on the normality of the data.

9. (p18, line 12) Kaplan-Meier method will be used to describe the risk of relapse, which requires data on time to event (ie time to relapse). But why "a similar method will be used to study the time to first relapse"(line 18)?

We rewrote the statistical analysis section and edited this sentence.

10. (p19, line 13) The study was approved by the Ethics committee on May 17, 2018. Please state whether the study is ongoing? If it is ongoing, what is the date of the study?

The trial is currently ongoing. Inclusion started in April 2019 and are planned to be completed by April 2021. Given the 2-year follow-up in the trial, the expected completion date is now April 3, 2023. This has been added to the manuscript.

VERSION 2 – REVIEW

REVIEWER	Elisabeth Hodson Centre for Kidney Research The Children's Hospital at Westmead Westmead, Westmead, NSW 2145, Australia
REVIEW RETURNED	17-Jun-2020

GENERAL COMMENTS	I have read the updated draft manuscript and believe that the authors have answered all my questions about the trial protocol. I have no further comments and believe that the manuscript is ready for publication.

REVIEWER	William E. Smoyer Nationwide Children's Hospital / The Ohio State University USA

REVIEW RETURNED	23-Jun-2020

GENERAL COMMENTS	The authors have done a very nice job of responding to the reviewers' concerns, and the described trial is now notably improved. I have just a few additional minor suggestions. P35/L54 - Please add FRNS as a key word to be more accurate about the trial. P42/L25 - Please clarify acceptable ages for inclusion... You have no upper age limit, so would you allow this to become a primarily adult study if recruitment were better among adults than children? If not perhaps you could limit the percentage or number of adults eligible to be recruited into the trial. P45/L43 - Since relapse is your primary study outcome, please clarify in the text if one or more first-Am urine dipstick results or urine protein/creatinine readings will be required for determining relapse... Even better would be to define more explicitly on P43/L40 your precise definition of a relapse.. i.e. three consecutive fist-Am urine protein/creatinine values above your threshold vs. one single abnormal reading? Also, will you require edema to be present to declare a relapse or not?

REVIEWER	Abhik Das RTI International, USA
REVIEW RETURNED	22-Jun-2020

GENERAL COMMENTS	My question in the previous review regarding inconsistency between the sample size calculations and the stated primary outcome has been addressed. However, I still have lingering concerns about the large effect size (50% reduction), which does not seem to be justified by any literature. In addition, while some concerns about the analysis plan have been addressed in the response letter, the overall analysis plan description in the manuscript itself still remains very brief. These are mainly issues with the protocol itself and not necessarily with this manuscript that describes the protocol. One minor issue: the last sentence in the Abstract Introduction: Should it be "addition" instead of "association"?

REVIEWER	LM Ho The University of Hong Kong
REVIEW RETURNED	15-Jun-2020

GENERAL COMMENTS	Thank you for the responses to the reviewers' comments. It is much clearer than the previous version. The revised statistical methods are consistent, but how to conduct the multiple imputation and how the distribution of the data determines the use of log-transform t-test or a Mann-Whitney test deserve, if possible, more explanation. I do not have any other comments.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 4
Reviewer Name

LM Ho

Institution and Country

The University of Hong Kong

Please state any competing interests or state ‘None declared’:
None

Please leave your comments for the authors below
Thank you for the responses to the reviewers’ comments. It is much clearer than the previous version. The revised statistical methods are consistent, but how to conduct the multiple imputation and how the distribution of the data determines the use of log-transform t-test or a Mann-Whitney test deserve, if possible, more explanation. I do not have any other comments.

We would like to thank the reviewer for this kind comment. The multiple imputation will be performed using a fully conditional specification (FCS) method as implemented in the proc mi in SAS. Considering the choice between a t-test or a Mann-Whitney, we will assess the distribution of the variables (and their log-transformation) visually and test it using a Kolmogorov-Smirnov test. If the normality of the distribution is rejected, then, a non-parametric test (Mann-Whitney) will be used, else the parametric log –transform t-test will be used.

Reviewer: 1
Reviewer Name

Elisabeth Hodson

Institution and Country

Centre for Kidney Research
The Children's Hospital at Westmead
Westmead, Westmead, NSW 2145, Australia
Please state any competing interests or state ‘None declared’:
None declared

Please leave your comments for the authors below
I have read the updated draft manuscript and believe that the authors have answered all my questions about the trial protocol. I have no further comments and believe that the manuscript is ready for publication.

We would like to thank the reviewer for reviewing our manuscript.

Reviewer: 3
Reviewer Name

Abhik Das

Institution and Country

RTI International, USA

Please state any competing interests or state ‘None declared’:
None declared
Please leave your comments for the authors below
My question in the previous review regarding inconsistency between the sample size calculations and the stated primary outcome has been addressed. However, I still have lingering concerns about the

large effect size (50% reduction), which does not seem to be justified by any literature. In addition, while some concerns about the analysis plan have been addressed in the response letter, the overall analysis plan description in the manuscript itself still remains very brief. These are mainly issues with the protocol itself and not necessarily with this manuscript that describes the protocol. One minor issue: the last sentence in the Abstract Introduction: Should it be "addition" instead of "association"?

We thank the reviewer for his review and for his help in improving our manuscript. We agree that the effect size chosen is important. Although no published study reports the effect of the used of IV immunoglobulin in association with RTX in this indication, the choice of this effect size was based on preliminary unpublished data and on the clinical agreement between the investigators that we were only interested in demonstrating a substantial decrease in the risk of relapse given the burden of the monthly IVIG infusions.

Reviewer: 2
Reviewer Name

William E. Smoyer

Institution and Country

Nationwide Children's Hospital / The Ohio State University

USA

Please state any competing interests or state ‘None declared’:
none declared

Please leave your comments for the authors below
The authors have done a very nice job of responding to the reviewers' concerns, and the described trial is now notably improved. I have just a few additional minor suggestions.

We would like to thank the reviewer for his helpful comments and for reviewing our manuscript.

P35/L54 - Please add FRNS as a key word to be more accurate about the trial.

We added FRNS as key word as requested.

P42/L25 - Please clarify acceptable ages for inclusion... You have no upper age limit, so would you allow this to become a primarily adult study if recruitment were better among adults than children? If not perhaps you could limit the percentage or number of adults eligible to be recruited into the trial.

Although we did not limit the number of adult patients, we only included 2 adult centers, which is minimizing the risk of including a high proportion of adult patients. We have now included more than half of the patients and only 1 was included in an adult center.

P45/L43 - Since relapse is your primary study outcome, please clarify in the text if one or more first-Am urine dipstick results or urine protein/creatinine readings will be required for determining relapse... Even better would be to define more explicitly on P43/L40 your precise definition of a relapse.. i.e. three consecutive fist-Am urine protein/creatinine values above your threshold vs. one single abnormal reading? Also, will you require edema to be present to declare a relapse or not?

We require the first-AM urine dipstick result to be confirmed by one urine protein/creatinine reading. This is now clearly specified in the manuscript: *“proteinuria will be evaluated one a week using a first-AM urinary dipstick until 12 months after rituximab injection and once every two weeks between 12 and 24 months. If the results are positive, a confirmatory urine analysis will be carried out in laboratory.”*

We do not require the presence of edema to define relapse. This is now clearly specified in the manuscript as follow:*" No clinical manifestation is requested to define relapse."*